Supplementary Methods

Flow cytometry

Exosomes (10 µg of protein) were bound to 5 µg aldehyde surface latex beads (Invitrogen) for 1 hr at room temperature. Bound exosomes were spun down and incubated in FACS permeabilization buffer. The unoccupied sites were saturated with vesicle free fetal calf serum and the exosomes were incubated with primary antibodies or control isotype for 1 hr at room temperature. The exosomes were spun down and incubated with FITC-conjugated secondary antibodies for 30 min at room temperature. The staining was analyzed on the FITC-channel by flow cytometry.

Supplementary Figures

Figure. 1

1A. Immunoprecipitation of EDTA eluates from cells expressing AnxA2WT-GFP in the absence and presence of A232187 with Anti-AnxA2 antibodies **1B.** NIH 3T3 cells expressing AnxA2-GFP were stimulated with ionophore and treated with 100 µM sodium orthovanadate. The biotinylated extracts were immunoblotted with GFP antibody and the blots were reprobed with Na,K-ATPase antibody for loading control. **1C.** Immunoprecipitation of TI fractions from ionophore-stimulated cells expressing AnxA2Y23E-GFP, AnxA2Y23F-GFP and empty GFP vector with GFP antibody and immunoblotting with AnxA2 antibody. For negative control, immunoprecipitation with a non-specific antibody was used. **1D.** Immunoprecipitation of the TI fractions from unstimulated and ionophore-stimulated cells expressing AnxA2WT-GFP and immunoblotting with phosphotyrosine antibody. The endogenous levels of AnxA2 in the input fractions were indicated in the lower panel

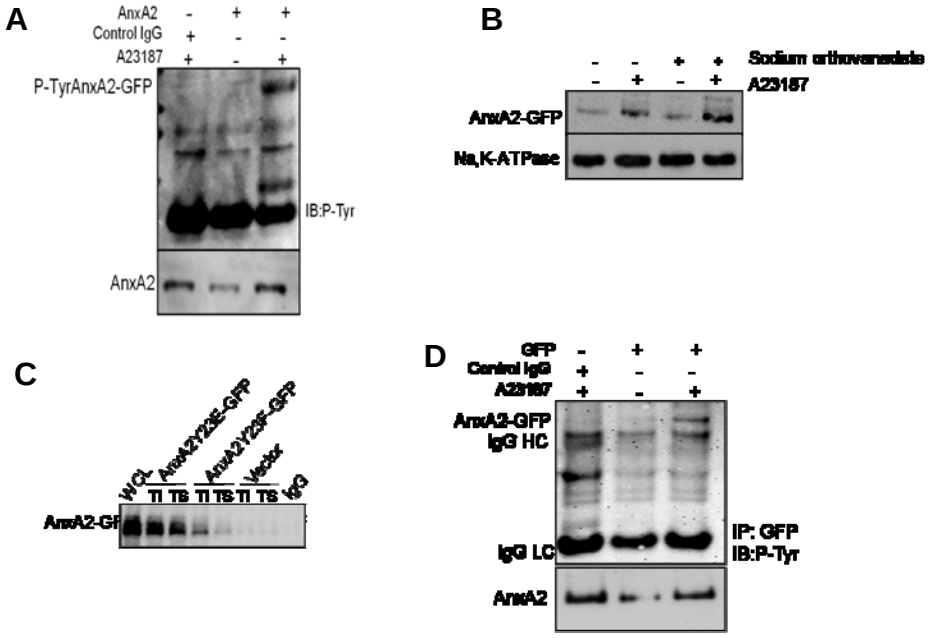

Figure. 2

Endocytosis of CTXB and transferrin in the presence of filipin.

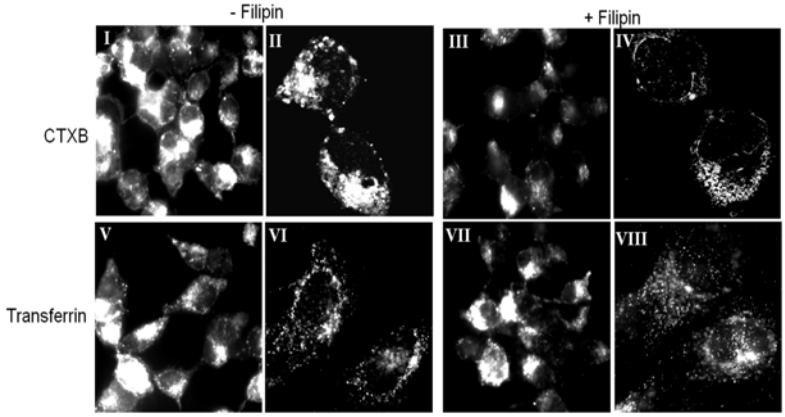

Figure. 3

Exosomal expression of Wild-type AnxA2 and Tyr-23 phosphorylation mutants. **(A-J)** Exosomes produced by ionophore-stimulated cells for 4 hr were coated on latex beads, labeled with the indicated antibody and subjected to flow cytometry analysis. Isotype controls are represented by broken lines and the continuous lines indicate staining by specific antibodies. Flow cytometry analysis of exosomes

collected from cells treated with the ionophore, for the expression of plasma membrane markers, CD45 and Na, K-ATPase, for the surface expression of exosomal markers CD81 and CD63. Staining of exosomes for the expression of endogenous AnxA2,

AnxA2WT-GFP and AnxA2Y23E-GFP in ionophore-stimulated cells. Exosomes were depleted of Ca^{2+} by treatment with EDTA and later stained for AnxA2.